Biochemical Pharmacology, Vol. 38, No. 9, pp. 1385–1388, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00

COMPARATIVE PHARMACOKINETICS OF NEW ANTI-HIV AGENTS: 2',3'-DIDEOXYADENOSINE AND 2',3'-DIDEOXYINOSINE

John W. Russell and Lewis J. Klunk

Department of Metabolism and Pharmacokinetics,
Bristol-Myers Co., Wallingford, CT 06492, U.S.A.

(Accepted 14 February 1989)

A number of 2',3'-dideoxynucleosides have been shown to inhibit the in vitro infectivity and cytopathic effect of the human immunodeficiency virus (HIV) [1]. These compounds, as their 5'-triphosphates, inhibit viral reverse transcriptase by competing with the natural substrate at the same binding site on the enzyme [2]. Dideoxynucleoside triphosphates can also be incorporated into growing DNA chains which then blocks further DNA elongation because they lack the 3'-hydroxyl group required for further polymerization [1]. Among these nucleosides, 2',3'-dideoxyadenosine (ddA) and 2',3'-dideoxyinosine (ddI) show promising in vitro activity [3]. Because adenosine is rapidly converted to inosine by adenosine deaminase [4], the in vivo conversion of ddA to ddI was studied to determine suitability of measuring plasma levels of ddI and to assess the bioavailability and pharmacokinetics of ddA. This report describes and compares the pharmacokinetics of ddA and ddI in the mouse.

MATERIALS AND METHODS

Materials. 2',3'-Dideoxyadenosine, 2',3'-dideoxyinosine, and 2',3'-dideoxy-2',3'-didehydrothymidine (d4T) were obtained from the Bristol-Myers Co. (Wallingford, CT). The purity of these compounds was greater than 97%. HPLC grade acetonitrile, deionized-distilled water, and methanol were obtained from American Burdick & Jackson (Muskegon, MI). All other chemicals were analytical grade or better. Male CD-1 (ICR) mice (20 ± 1 g) used for these studies were obtained from Charles River Breeding Laboratories (Wilmington, MA).

Dosing and sample collection. For the pharmacokinetic studies, both ddA and ddI (5.5 mg/ml in deionized distilled water) were administered orally at a volume of 5 ml/kg by gastric intubation and intravenously as a 0.1 ml bolus injection into the tail vein to provide a dose of 27.6 mg/kg. Blood samples were taken from the posterior vena cava of three anesthetized mice at each of the time points shown in Fig. 1. The plasma was separated by centrifugation and stored frozen until analyzed for ddI.

To estimate the in vivo conversion of ddA to ddI, six mice were each given a single i.v. dose of 25 mg/kg of ddA. Blood was taken from three of the mice 5 min after dosing and from the remaining mice 10 min after dosing. Immediately after collection, the blood samples were thoroughly mixed with 0.5 ml of 0.05 M $HgCl_2$ to inhibit adenosine deaminase. The plasma was separated from each sample and analyzed for the presence of ddA.

Analytical methodology. Plasma, 0.25 ml, was added to 0.5 ml of 0.05 M $HgCl_2$ which contained 4.0 μg/ml of internal standard, d4T. The mixture was briefly vortexed and then transferred to a C18 SEP-PAK cartridge. The sample was pulled through the cartridge under vacuum, and the cartridge was washed with 5 ml of distilled-deionized water. Both ddI and d4T were eluted with 2 ml of methanol:water (40:60) mixture. Standards were prepared daily by adding known amounts of ddI to control mouse plasma to give final concentrations ranging from 0.5 to 20 μg/ml.

The samples were analyzed by HPLC by injecting 25-75 μl of the extract onto a Zorbax C8 column and monitoring absorption at 254 nm with a Waters model 481 UV detector. The flow rate was 1.0 ml/min and the mobile phase was sodium phosphate buffer (0.01 M, pH 6.8):methanol:acetonitrile (86:6:8). The retention times of ddI and d4T were 6.2 and 7.6 min respectively. Detector response was based on the measurement of peak height by using a Hewlett Packard model 3392A recording integrator. The ratio of the peak height of ddI to that of the internal standard was determined for a series of standards. These data were then used to prepare a calibration by a linear regression analysis. Calculation of the concentration of ddI in unknown samples was determined from the regression equation by using the peak height ratio. The lower limit of quantitation for this study was 0.4 μg/ml.

Pharmacokinetic calculations. The elimination rate constant (ß) was determined from the slope of the terminal linear portion of the ln concentration (C) versus time (t) curve. The slope was determined by a least squares linear regression. The elimination half-life ($T_{\frac{1}{2}}$) equals ln (2)/ß. The area under the plasma concentration versus time curves (AUC) were calculated by the trapezoidal rule from zero time to 45 min for ddA and 40 min for ddI. Bioavailability (F) was calculated from the plasma AUC data by using the following equation:

$$F = \frac{AUC_{oral} \cdot Dose_{i.v.}}{AUC_{i.v.} \cdot Dose_{oral}} \times 100$$

The total clearance, Cl_T, was calculated from the dose divided by the $AUC_{0\rightarrow Cn,i.v.}$ The apparent volume of distribution (V_d) equals Cl_T multiplied by ($T_{\frac{1}{2}}$/0.693).

RESULTS AND DISCUSSION

Initial studies indicated that ddA was rapidly converted in vitro in mouse plasma to ddI with a half-life of 20 min. The rate of this conversion was dependent on the extent of hemolysis of the plasma samples, presumably because of a higher concentration of adenosine deaminase in the erythrocytes. The half-life of the conversion was found to range from 45 min in carefully prepared plasma samples to 6 min in plasma prepared from slightly hemolyzed blood.* The study to characterize the in vivo conversion of ddA to ddI showed that high plasma concentrations of ddI were observed within 5 min after i.v. administration of 25 mg/kg of ddA, whereas there were no detectable quantities of ddA at this time. Therefore, the pharmacokinetic properties of ddA were determined by measurement of ddI in plasma.

Semilog plots of the mean plasma concentration of ddI after administration of

*Unpublished results.

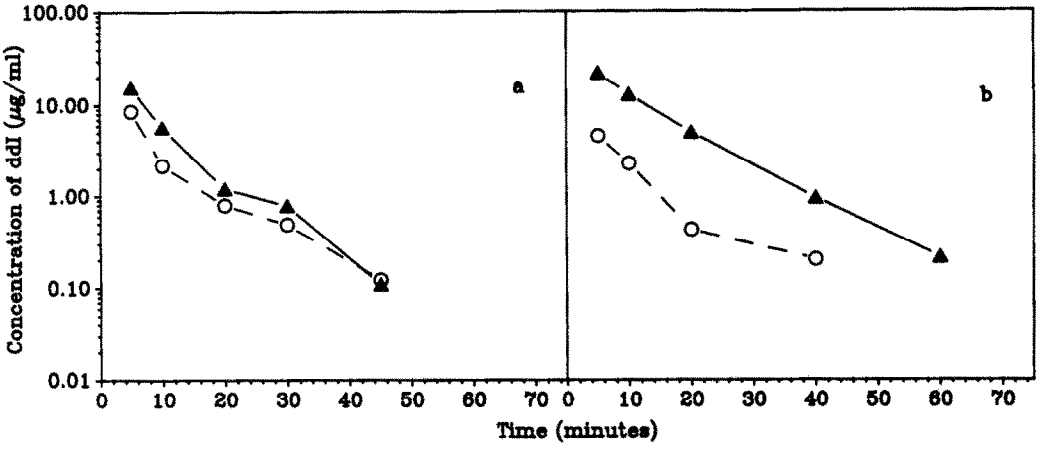

Fig. 1. Mean plasma concentration of ddI measured after oral (0--0) or i.v. (▲—▲) administration of (a) ddA (27.6 mg/kg) and collecting blood samples (three mice per time point) 5, 10, 20, 30, 45 and 60 min after dosing, and (b) ddI (27.6 mg/kg) and collecting blood samples 5, 10, 20, 40, 60 and 90 min after dosing. Plasma concentrations below the quantitation limit are not shown.

either ddA or ddI are shown in Fig. 1. A summary of the pharmacokinetic parameters is shown in Table 1. Both compounds were absorbed rapidly after oral administration with maximum plasma concentrations of ddI occurring by 5 min after dosing. Plasma concentrations decreased rapidly with an elimination half-life (i.v.) for ddI of approximately 6 min after administration of ddA and 8 min after administration of ddI. These values are about one-tenth of that reported for 2',3'-dideoxycytidine (ddC) (69 min) [5] and about one-third of that found for AZT (Zidovudine) (23 min).* The absolute bioavailability of ddA (38%), based on the measurement of ddI in plasma, was about three times greater than that calculated for ddI (13%). The bioavailability of ddA in the mouse was slightly greater than that for the related pyrimidine nucleoside analogue, ddC (30%), and less than half of that determined for AZT (100%).*

Both ddA and ddI are susceptible to degradation under acid conditions and probably undergo some intragastric degradation in the stomach after oral administration. This could account for the less than complete oral bioavailability of both drugs in the mouse. The 3-fold difference in the bioavailability between these compounds could be attributed to the fact that ddI is more unstable in acid than ddA.

The results of this study indicate that ddA may be serving as a prodrug which is converted rapidly to ddI. ddA and ddI have been shown to be equipotent in vitro in inhibiting the infectivity and cytopathic effect of HIV [3]. However, the observed equipotency of these compounds could be due to rapid in vitro conversion of ddA to ddI in the assay system. Radiolabeled ddA and ddI have been shown to generate identical metabolite profiles when incubated in vitro with Molt-4 cells, and pathways were proposed which could account for the formation of ddATP from either ddA or ddI through a common intermediate, ddIMP [6]. The data from this study indicate that ddI is present in high concentrations in plasma after administration of ddA or ddI. Therefore, the selection of either ddA or ddI for development as an anti-HIV agent may

*Unpublished results.

Table 1. Summary of pharmacokinetic parameters for ddA* and ddI

		C_{max} (μg/ml)	t_{max} (min)	$T_{\frac{1}{2}}$ (min)	AUC (μg·min/ml)	Cl_T (ml/min)	V_d (ml)	F (%)
ddA	i.v.	--	--	5.9	196.2	2.8	24	--
	oral	8.6	5	--	74.5	--	--	38%
ddI	i.v.	--	--	8.4	376.9	1.5	18	--
	oral	4.6	5	--	50.3	--	--	13%

*Measured as ddI.

be appropriate. The only potential advantage for ddA would be its greater, but still relatively low, bioavailability; however, the oral bioavailability of ddI could be enhanced by overcoming its acid instability through appropriate formulation techniques.

ACKNOWLEDGEMENTS

The assistance of M. Bies, C. Ferraro, D. Isgar and Dr. Y. Tsai in dosing animals and collecting blood samples for these studies and of Rosemary Hall for preparing the manuscript is gratefully acknowledged.

REFERENCES

1. Mitsuya H and Broder S, Strategies for antiviral therapy in AIDS. Nature 325: 773-778, 1987.
2. Chen MS and Oshana SC, Inhibition of HIV reverse transcriptase by 2',3'-dideoxynucleoside triphosphates. Biochem Pharmacol 36: 4361-4362, 1987.
3. Mitsuya H and Broder S, Inhibition of the in vitro infectivity and cytopathic effect of human T-lymphotropic virus type III/lymphadenopathy-associated virus (HTLV-III/LAV) by 2',3'-dideoxynucleotides. Proc Natl Acad Sci USA 83: 1911-1915, 1986.
4. Frederikson S, Specificity of adenosine deaminase toward adenosine and 2'-deoxyadenosine analogs. Arch Biochem Biophys 113: 383-388, 1966.
5. Kelley JA, Litterst CL, Roth JS, Vistica DT, Poplack DG, Cooney DA, Nadkarni M, Balis FM, Broder S and Johns DG, The disposition and metabolism of 2',3'-dideoxycytidine, an in vitro inhibitor of human T-lymphotrophic virus, Type II infectivity in mice and monkeys. Drug Metab Dispos 15: 595-601, 1987.
6. Ahluwalia G, Cooney DA, Mitsuya H, Fridland A, Flora KP, Hao Z, Dalal M, Broder S and Johns DG, Initial studies on the cellular pharmacology of 2',3'-dideoxyinosine, an inhibitor of HIV infectivity. Biochem Pharmacol 36: 3797-3800, 1987.